

Why *Cannabaceae* is an interesting family?

Cannabis sativa L.,

Hemp
 $2n = 20$

♀ haploid genome size = 818 Mb
♂ haploid genome size = 843 Mb
 $Y > X$

Female plant has $2n = 20(XX)$
Male plant has $2n = 20(XY)$



Humulus lupulus L.,

Common hop
 $2n = 20$

♀ haploid genome size 2960 Mb
♂ haploid genome size 2730 Mb,
 $Y < X$

Female plant has $2n = 20(XX)$
Male plant has $2n = 20(XY)$



Humulus japonicus L.

Japanese hop
 $2n = 16/17$

♀ haploid genome size = 1568 Mb
♂ haploid genome size = 1722 Mb
contains XY_1Y_2

$Y_1 < X > Y_2$
Female plant has $2n = 16(XX)$
Male plant has $2n = 17(XY_1Y_2)$

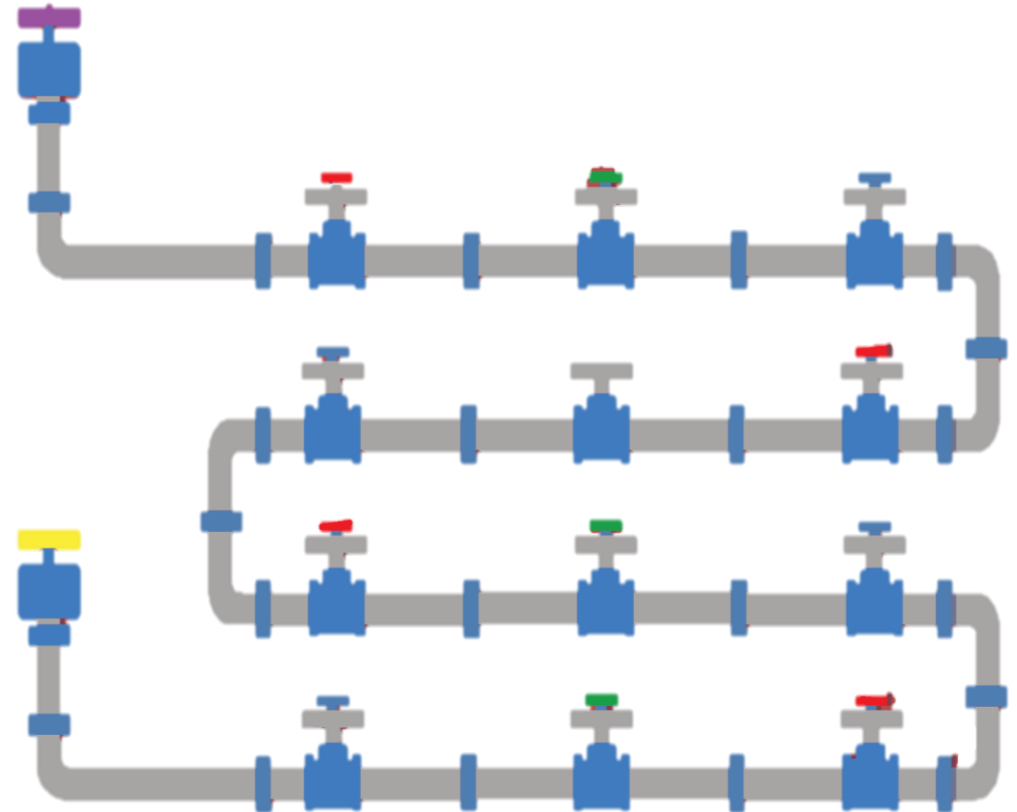


Analysis of repeatomes in Cannabaceae family

The work was supported by **RFBR № 20-316-70018\19** and done by J. Bocharkina, O. Razumova, G. Karlov

What was *our pipeline*?

1. DNA extraction (CTAB method);
2. Preparing DNA libraries according to the instructions of commercial kits;
3. Illumina MiSeq sequencing;
4. Quality control;
5. Filtering;
6. Interlacing;
7. RepeatExplorer2 analyzing;
8. RepeatMasker identification;
9. Pairwise scatterplotting;
10. PCR



What about repeatomes analysis?

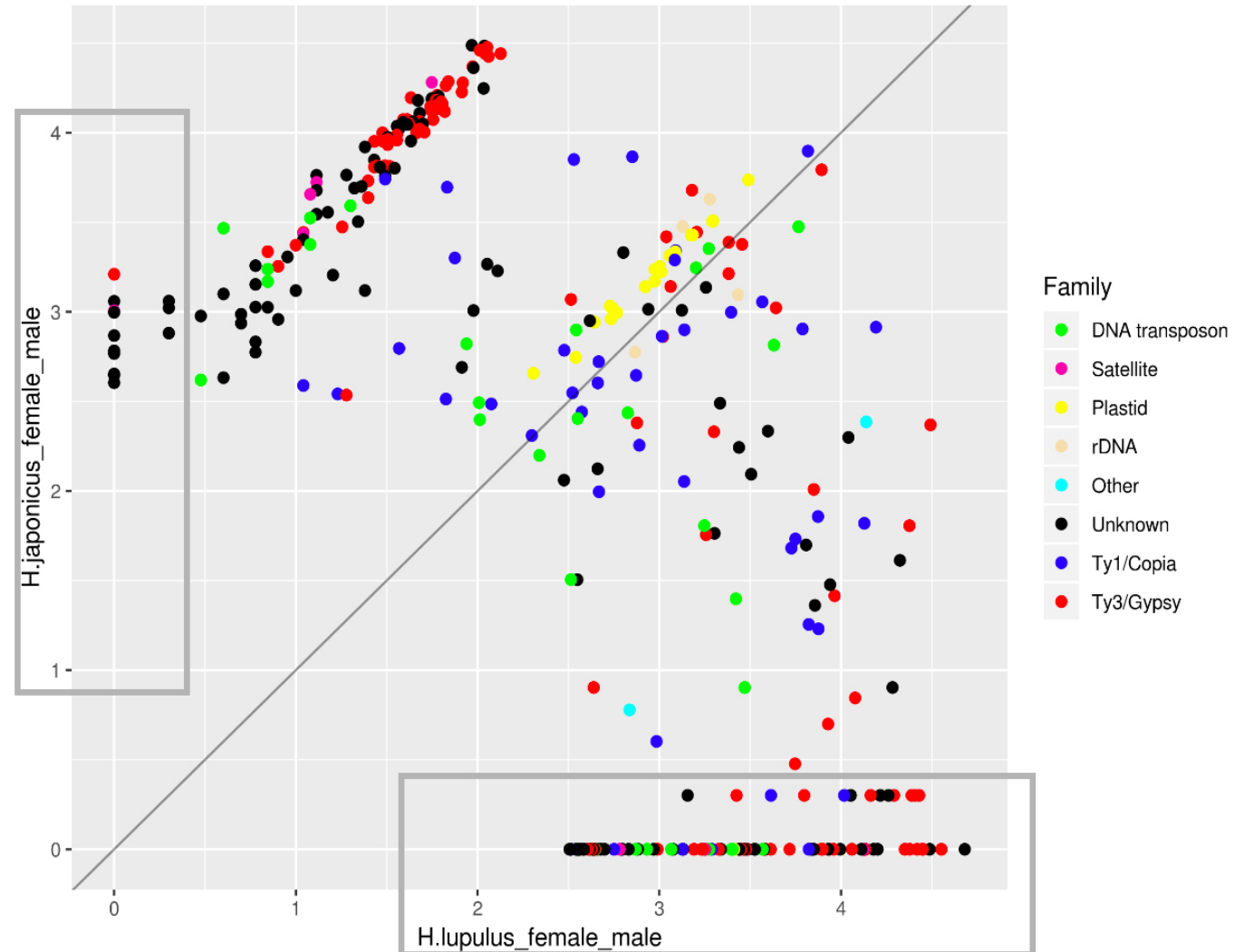
1) The majority of species-specific sequences for both genomes are non-classified repeats-families. And a large amount of known species-specific repeats related to the Ty3/Gypsy family (~8%).

2) Finally, **88** species-specific clusters for the common hop and **10** species-specific clusters for Japanese hop were identified. The highest-copy clusters were selected as candidates for the creating of species-specific markers.

3) The highest-copy clusters were selected as candidates for the development of species-specific markers:

For common hop, the clusters: CL2 (Ty3 / Gypsy, Tekay), CL10 (Ty3 / Gypsy, Retand), CL59 (Satellite), CL117 (Ty1 / Copia, Angela);

For Japanese hop clusters: CL233 (Ty3 / Gypsy - Retand), CL263 (Satellite).



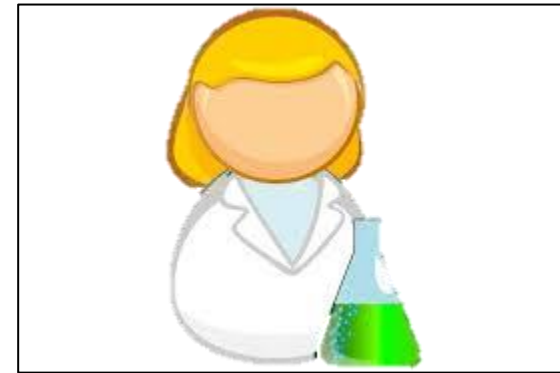
PCR analysis

Primers were created and, finally, we had 17 pairs of primers. We decided to check our primers with doing PCR and electrophoresis It was shown that most of the primers are not species-specific.

1) Universal for *Cannabaceae* family Cl 83-2, Cl 2, Cl 111(polymorphism for *H. lupulus* and *H. japonicus*), Cl16, Cl 4(sex polymorphism *H. japonicus*), Cl 35, Cl 107

2) Universal for *Humulus lupulus* and *Humulus japonicus*: Cl 83-1, Cl154(polymorphism for both *Humulus* species), Cl 67, Cl 69, Cl 23

3) Species specific: Cl 62(sex polymorphism for *H. japonicus*), Cl 51 (specific for *H. japonicus*), Cl 6(sex polymorphism for *H. japonicus*), Cl72 (sex polymorphism for *H. japonicus*), Cl77 (specific for *H. lupulus*)



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